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FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 695 Huntington Ave., Boston, Mass.

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NUTRITION REVIEWS

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FALSE ESTIMATES OF ADULT REQUIREMENTS

Several papers have appeared in recent years which present the results of balance studies made on adult groups consuming their usual diets (H. McKay *et al.*, *J. Nutrition* **24,** *367* (*1942*); P. H. Roberts, C. H. Kerr, and M. A. Ohlson, *J. Am. Dietet. Assn.* **24,** *292* (*1948*); Ohlson, *Fed. Proc.* **11,** *581* (*1952*); and others). From such data, the mean intakes of the nutrients which are required to maintain balance in the average individual of the group may be determined. It appears that such estimates are not being correctly distinguished from nutritional requirements. It is the purpose of this paper to indicate my conviction that *these studies have no significance as estimates of adult requirements*, and that incorrect interpretations will lead us into serious difficulties.

For purposes of discussion we will assume that we are conducting such a study on the relation of nitrogen intake to nitrogen balance. Any other nutrient in which balance studies are appropriate would do as well. The group under study is well fed by modern standards. The average intake is 100 g. of protein (16 g. of nitrogen) per day. Furthermore, the *average* intake of each individual is also 100 g. per day, but as is usual the daily or weekly intakes fluctuate widely. As the data are collected over a one-week period we find that the measured intakes vary from 75 to 125 g. of protein per day (12 to 20 g. of nitrogen).

Now it is well known that when the dietary intake of a nutrient falls below the usual intake, the individual goes into a negative balance for some period of time. Similarly, when the intake rises above the usual intake, a positive balance is observed. However, a change from the mean intake of equal degree either up or down does not result in equal positive and negative balances. The body utilizes nutrients less efficiently as the intake rises. Thus a considerable increase in intake may be required to produce a relatively small positive balance. On the other hand, when the intake falls the body loses some of the stored nutrient and the negative balance will be larger than the change in intake. This negative balance will not persist, however, unless the intake falls below the actual minimum requirement. Under the stimulus of the loss, the efficiency of the body increases and eventually balance is restored at the lower level of intake. The mechanisms involved are of great interest but not well known, and they are not pertinent to this discussion. The facts are clear as far as protein is concerned and must logically hold for other less well studied nutrients where balance studies are appropriate. The maintenance of balance in the body is like pumping water up a hill. If you want to maintain the level high you have to pump harder. It takes much more energy to raise it the last ten feet than the first ten from the source of supply.

To return to the illustrative example, we have a large group of adults with a mean intake of 16 g. of nitrogen per day. They show a random distribution about this mean, with some individuals consuming as much as 20 g. and others as little as 12 g. during the week the data are collected. This variation is not so great as the spread of data usually collected in the field (McKay *et al.*, *loc. cit.*). Actual studies on the weekly intakes of individuals show a variation of about this magnitude (J. Yudkin, *Brit. J. Nutrition* **5,** *177* (*1951*)). Plotting the number of persons at each level of intake we will obtain a curve approximating

the well known "normal" curve. There will be few values at the extremes of 12 and 20 g. of nitrogen intake and the numbers will increase as we approach the mean from either side.

What do we expect the nitrogen balances to show? In view of the facts discussed above, that lowering the intake results in negative balances larger than the change in intake and the opposite when the intake is raised, and that the degree of negative or positive balance is dependent upon the change in intake, it would be expected that as expected, values to the left of the mean are negative while those to the right are positive. Two more or less normal distributions are obtained. In fact, the intake data fit the normal curve so well that the theoretic distribution is the one shown. The nitrogen balance maximum (actually minimum) falls to the left of the intake maximum. Even the skewness of the nitrogen balance curve might have been anticipated from the above considerations. It should be recognized that the position of the ordinates of the two curves is entirely arbitrary.

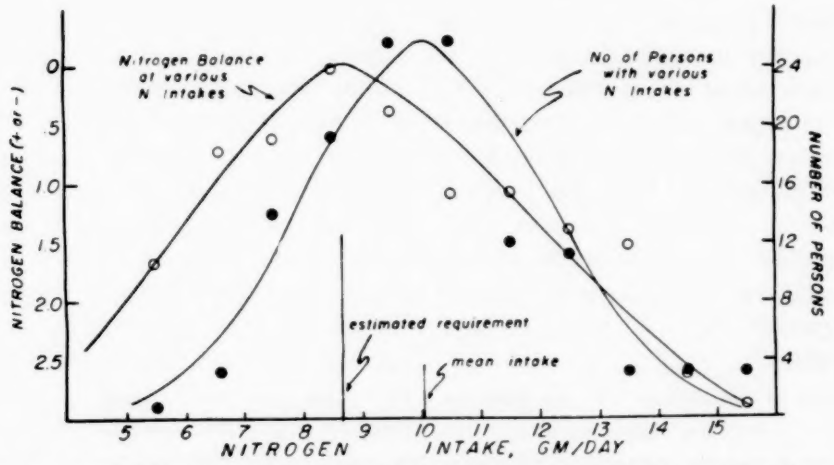

FIG. 1. The two curves show the number of individuals consuming the various levels of nitrogen and the nitrogen balance (either + or −) at these nitrogen intakes. Data taken from the paper by H. McKay *et al.*, *J. Nutrition* **24**, *367 (1942)*.

the variations in nitrogen balance from zero—either positive or negative—would also show a normal distribution, but that the curve would be displaced toward the left (lower intakes) when compared to the distribution of intakes. The intake giving a balance of zero should thus be somewhat less than the mean intake of the group.

Data from the literature appear to behave as anticipated. McKay *et al.* (*loc. cit.*) presented dietary intakes and balances of nitrogen, calcium and phosphorus for 124 women consuming their usual diets. In Fig. 1 the two distributions for the data on nitrogen intake and nitrogen balance are shown. As explained above, nitrogen balance is plotted without regard to sign but,

McKay *et al.* also estimated the mean intake required to maintain balance in this group. This is at 8.59 g. of nitrogen per day as indicated on the figure. It is approximately 1.5 g. less than the mean intake and approximates the maximum of the nitrogen balance curve as drawn. Similar plots for the data on calcium and phosphorus which cannot be shown here present a very similar picture. The data on calcium do not conform so well, but this would be expected since the adjustment of the body to new calcium intakes must occur very slowly.

If the above considerations are true, it is apparent that the average requirement to maintain balance in any reasonably stable adult group consuming diets above their

actual minimum requirement *is determined by the average intake of the group*. The situation will not be changed whether the average intake of the group is 70, 100, or 150 g. of protein per day. No end is in sight if we should base dietary recommendations upon such data.

It should be recognized that no person consuming freely selected diets is "in balance" except as an average situation. In practical terms, "in balance" means that the sum of the negative and positive balances is zero over the long run. Actually a person is in either negative or positive balance most of the time. He would be at zero balance rather rarely and this would depend entirely upon how exactly the measurements were made. In view of the dynamics of the situation we might expect that several days of positive balance would be required to compensate for one day of negative balance and that he would be in positive balance most of the time.[1] Similarly, we expect and find that about half, or perhaps somewhat less, of a group consuming their usual diets are in negative balance and the remainder in positive balance. Thus it is certain that most of the values obtained over a day or week study period *do not* represent the usual diets or average diets of the persons studied. If they did, it is clear that we would find about half of our population withering away due to protein loss while the remainder would gradually assume giant proportions. Neither a continuous positive nor negative balance is compatible with health in the adult state for any considerable period of time.[1] Except in unusual cases, the finding of a negative or positive balance of any real degree must be considered evidence that the diet is not the usual diet of that person.

Perhaps it should be pointed out that this article is not designed, nor are we inclined, to discredit balance studies as a useful experimental technic. There are many conditions where the dietary intake is low, the intakes are varied, the status of the individual under study is not stable, etc., where useful data are obtained. Indeed, balance studies may be about the only method available. However, the balance technic in the study of an apparently stable population group consuming their usual diets which are well above minimum requirements is not a useful application since the results have no apparent relationship to either nutritional status or nutritional requirements.

D. M. Hegsted
Associate Professor of Nutrition
Harvard School of Public Health and the Department of Biochemistry,
Harvard Medical School

[1] We ignore here the fact that a slight positive balance to allow the growth of hair, nails, losses from skin, etc., is the steady state in the adult. Ordinarily the study will not be sufficiently exact to differentiate this from a balance of zero.

LIPOTROPIC SUPPLEMENTS IN FATTY CIRRHOSIS

The evidence concerning the necessity of methionine and choline in the therapeutic regimen for patients with cirrhosis or hepatitis has been reviewed on several occasions (*Nutrition Reviews* **9,** *247 (1951)*; **10,** *12, 79 (1952)*). Although immutable conclusions cannot yet be reached, the data at hand strongly suggest that little or no benefit is to be derived from the use of these agents in patients whose livers are not primarily fatty, or in patients able to eat an adequate diet regardless of the degree of fatty infiltration. In view of the relatively small number of adequate studies of this problem, the appearance of more recent evidence is noteworthy.

In an attempt to evaluate the effect of choline supplementation upon fatty infiltration in the livers of alcoholic patients, J. Post, J. G. Benton, R. Breakstone, and

J. Hoffman (*Gastroenterology* **20,** *403* (*1952*)) have observed 17 patients in 19 study periods. Investigation of these patients was carried out by means of biweekly liver function tests, liver biopsies at three- to fifteen-week intervals, and clinical observations. Thirteen of the patients composed one group who received a diet of approximately 2000 calories containing 75 g. of protein supplemented with B-complex vitamins. A second group consisted of 2 patients who were offered the same diet and vitamins, but who ate poorly and drank heavily of alcohol obtained by "clandestine measures." A third group of 4 patients received the diet plus choline (17 g. of choline bicarbonate daily for 3 patients, 4 g. daily for one patient).

Except for the second group all patients gained weight and showed improvement clinically and in liver function. Marked improvement in the fatty infiltration was also observed in these subjects, but no correlation was found between liver function tests and histology of the liver biopsies. By comparison, the patients who continued to drink showed little or no improvement by function test or biopsy.

Opportunity to study the effect of all three regimens on the same patient was afforded by three separate hospital admissions following periods of heavy drinking. On the first entry the subject continued to drink, ate poorly, and little benefit was noted. Four months later marked fatty infiltration was again present and the choline-supplemented regimen produced marked improvement. A third admission with similar findings eight months later allowed use of the diet without choline which also produced marked improvement.

The authors were unable to demonstrate any significant differences between the first and third group of patients in the rate or degree of change toward normal in liver function or histology. Despite the relatively small series of patients in the choline-treated group this study suggests that this lipotropic agent produces no benefit which is not obtained with the administration of an adequate diet.

In an accompanying report, W. D. Cayer and W. E. Cornatzer (*Gastroenterology* **20,** *385* (*1952*)) have again expanded their studies of phospholipid turnover (*Nutrition Reviews* **10,** *79* (*1952*)). As in previous studies, they found that the administration of single large doses of choline or methionine prior to dietary or other therapy resulted in an elevated phospholipid turnover as compared with that observed after therapy. Treatment in this study consisted of a 3500-calorie, 120- to 140-gram protein diet plus choline or methionine and resulted in a decrease in fat observed on serial liver biopsies in all except 3 of the 10 patients. Persistent fatty infiltration and no change in phospholipid turnover occurred in one patient in whom death followed shortly. A slight increase in fat in a second patient and no change in a third were reported, however, despite significant decreases in phospholipid turnover in these individuals.

Similar studies in 10 patients with cirrhosis without fatty infiltration revealed significant changes in phospholipid turnover in only 2. Patients with hepatitis responded similarly, and 6 of 7 had no change in phospholipid turnover.

While highly suggestive that fatty infiltration in alcoholic patients is due to a deficiency of choline or methionine, the conclusions of Cayer and Cornatzer cannot be final in view of the exceptional cases noted above. It appears that some patients with fatty infiltration do not respond to lipotropic agents while others demonstrate phospholipid turnover responses induced by lipotropic substances without correlated changes in liver fat. In addition, it can be said that some patients without demonstrable fatty infiltration reveal phospholipid changes consistent with an initial deficiency of lipotropic factors.

These investigations approach the problem of lipotropic agents in human liver disease in different ways. The tentative conclusions which they reach, however, are

not widely separated and the value of these agents would appear to be limited to the initial phases of therapy in patients with fatty infiltration, with little benefit to be derived thereafter when patients are able to ingest adequate diets.

KWASHIORKOR IN AFRICA

One of the most widespread nutritional disorders in tropical and subtropical areas is a syndrome at present ill defined. It is known by various names such as kwashiorkor, malignant malnutrition, polydeficiency disease, and infantile pellagra (*Nutrition Reviews* **4,** *52, 303 (1946)*; **6,** *270 (1948)*; **8,** *55, 161 (1950)*; **9,** *129, 225 (1951)*). As the result of a recommendation made by the joint Food and Agriculture Organization/ World Health Organization Expert Committee on Nutrition in October 1949, J. F. Brock and M. Autret visited a large number of territories in Africa and have presented a report (*Bull. World Health Org.* **5,** *1 (1952)*) of their observations and a critical analysis of the available literature.

In this report kwashiorkor is defined as a nutritional syndrome (or syndromes) found among indigenous Africans and composed of five essential features: retarded growth in the late breast-feeding, weaning, and postweaning ages; alterations in skin and hair pigmentation; edema; fatty infiltration, cellular necrosis, or fibrosis of the liver; and a heavy mortality when not treated. A variety of dermatoses, gastrointestinal disorders, mental apathy, and anemia are commonly associated with but not essential components of this syndrome. While the report of Brock and Autret is restricted to kwashiorkor in the preweaning and postweaning child in certain areas in Africa, they recognize that a similar syndrome occurs in other parts of the world and is often given the same name.

Growth retardation has been studied quantitatively in large numbers of infants and children in Kampala where kwashiorkor is frequent. Compared with accepted European standards, weight gain is normal during the first nine months of life when a deviation begins, showing the greatest retardation between the second and fourth years of life. The presence of edema may mask the severity of nutritional failure in infants suffering from this disease.

The pigmentary changes in the skin and hair is one of the early manifestations of the condition. There may be both a quantitative reduction in the amount of pigment normally present and qualitative changes as well. The term *dyspigmentation* has been used to express both of these changes. Pigmentation may be patchy or diffuse, the latter type being very difficult to distinguish from genetic hypopigmentation. Varying degrees of hair dyspigmentation commonly occur without alterations in texture. The reddish color of the hair responsible for the name of this syndrome (kwashiorkor means "red boy") is a common change. Normal hair color and texture return following treatment, but only in the new hair, with evidence of previous malnutrition remaining in hair which had developed prior to treatment.

The edema is almost invariably associated with hypoalbuminemia. Hyperglobulinemia is also frequently found, and the total serum proteins may approach normal values. Fatty infiltration of the liver is an extremely common postmortem finding. In some areas fibrosis without fatty change has been described. This may be due to differences in the carbohydrate supplements given to the very young infants. The mortality in untreated cases has varied from 30 per cent to 90 per cent or more.

The dermatoses frequently found in cases of kwashiorkor were first described by C. D. Williams (*Arch. Dis. Childhood.* **8,** *423 (1933)*) and the nonpellagrous origin of these lesions was recognized chiefly on the basis of geo-

graphic distribution. Later assumption of pellagrous origin led to use of the inappropriate term "infantile pellagra." Brock and Autret (*loc. cit.*) found that the dermatosis was not the same in all parts of Africa and concluded that the dermatoses were associated conditions having various etiologies.

Mental apathy is a very striking clinical feature in many cases. The presence of a mild normocytic or slightly macrocytic anemia is quite common. Atrophy of the acini of the pancreas and diminution of the enzymatic activity of the duodenal contents have been reported (J. N. P. Davies, *Lancet* **I,** *317 (1948)*).

The imperceptible transition from kwashiorkor to marasmus is noted. The "Mehlnährschaden" of A. Czerny and A. Keller ("Des Kindes Ernährung, Ernährungstörungen und Ernährungstherapie," *2d edition, Vol. 1 and 2, Leipzig and Vienna (1925, 1928)*) shows no dyspigmentation, but is otherwise quite similar. The term "malignant malnutrition" reflects the high mortality, but the favorable effects of treatment would argue against its use.

The nutritional origin of kwashiorkor is considered by Brock and Autret to be generally accepted. In reviewing the evidence on this point they have considered at length the dietary habits of African children. Breast milk, supplemented in some areas with carbohydrate gruels, is the sole food until after the age of 6 months. Supplementary foods given after that age are of the adult type and almost never include cow's milk. The portions of the adult meal which appear to be the most digestible and to require the least mastication are used. These foods are subjected to excessive cooking which further reduces their meager nutritive value. They consist of gruels made from cereals, especially manioc, corn, taro, and other tubers, fresh vegetables in season, and stews hardly ever including meat or fish. Even when the latter are present they are usually consumed by the breadwinner. The diets of pregnant and lactating women receive no special consideration.

Insufficient information concerning the food consumption in Africa makes accurate estimation of nutrient intake quite difficult, but certain tentative conclusions could be drawn from the general experience of doctors, missionaries and nurses. Generally it was found that kwashiorkor abounded in areas where the plantains, cassava, and tubers were the staple foods used almost exclusively and that the disease was practically unknown where milk, meat, fish, or beans were consumed regularly. Millet and rice appeared to occupy an intermediate position.

When available figures for the protein supplied by several of these foods per unit of 100 calories and the protein received when these foods were used to meet caloric requirements (with liberal allowances for both protein and calories received from breast milk) are tabulated it is clear that the diet of African children becomes deficient in protein at the age at which kwashiorkor occurs. Compared with milk, which supplies 5.4 g. of protein per 100 calories, millet supplies 2.9, rice 2.0, plantains 1.0, and cassava flour 0.44 g. of protein per 100 calories. The regions in which kwashiorkor is likely to occur are those in which the staple foodstuffs contain less than 2 g. of protein per 100 calories.

There is little doubt that kwashiorkor is due to a deficiency in the diet of some factor or factors ordinarily supplied by foods containing protein from animal sources or vegetable proteins of high biologic value. But the diets of African children are never deficient in a single factor. While the most obvious deficiency is that of methionine, it would be a mistake to conclude that this is the only deficiency, or even the most prominent one, since the role of interrelationships between amino acids and such vitamins as B_{12} and pyridoxine needs to be considered. However, it is well known that kwashiorkor cannot be cured by any combinations of vitamins that have been tried.

The remarkable therapeutic effect of milk protein in the form of dried skim milk nar-

rows somewhat the field of speculation. Furthermore, it is pointed out that the factors responsible for the dyspigmentation of the hair and fibrosis of the liver seen in 6-month-old infants fed exclusively at the breast are presumably lacking from the mother's milk.

Regarding the etiologic role of parasitism there is a relative paucity of information. It is unquestionable that kwashiorkor occurs in Johannesburg where the great majority of the population has never been exposed to malaria or other parasites. In the area surveyed, however, tropical parasitic diseases can be found in almost every case coming to autopsy. The age incidence of kwashiorkor corresponds to the age of relative absence of immunity to malaria, the commonest parasitic disease. Brock and Autret conclude that while tropical parasites are frequently contributory to the development of kwashiorkor, it is likely that the dietary factor is always dominant.

The epidemiologic relationship between primary carcinoma of the liver and kwashiorkor is accepted as strong evidence for dietary deficiency, especially of proteins, in both conditions. However, there is reason to believe that cirrhosis and hence primary carcinoma of the liver may not always be preceded by fatty infiltration. Furthermore, the supposition that kwashiorkor represents an early manifestation of cirrhosis is abandoned and the opinion is expressed that both diseases occur following prolonged deficiency of protein or of some factor closely related to protein. Protein deficiency appears to be a relatively permanent condition in the majority of inhabitants of the area surveyed.

In conclusion, the authors make several recommendations for the prevention of kwashiorkor. Since the provision of adequate supplies of animal proteins in these areas would require many years, their attention is focused largely upon improving the quality of the protein of vegetable origin in the diet. Cassava and similar foods give high yields of calories per acre and do prevent famines. Peanuts, soybeans and protein-rich varieties of millet are among the plants giving good yields of protein. The improvement of indigenous pulses and other foods familiar to these people is recommended instead of the introduction of new foods which might not be accepted. Besides these general measures for dietary improvement, more suitable supplementary foods for weanling infants are badly needed to replace the starchy gruels. Artificial "milk" from pulses or peanuts, which can be made at home, is suggested. By means of education and aroused interest some progress is being made.

An immediate need is for adequate provision of skim milk powder in hospitals and dispensaries for the treatment of infants sick with kwashiorkor, and another is dissemination of knowledge concerning the life-saving value of such treatment.

This report by Brock and Autret will be of great aid to those interested in the problems of kwashiorkor. The essentials of the clinical picture are clearly presented and separated from other manifestations of the syndrome whose variation from place to place has been a source of confusion. The dietary analysis, though admittedly crude and incomplete, has focused attention upon the important role of protein and its constituent amino acids. The complicated mechanisms involved in the development of this disease are obvious, and the directions in which further studies should be pursued become clear.

ALKAPTONURIA

The rare metabolic disorders, alkaptonuria and ochronosis, have been studied recently by W. J. Martin, L. O. Underdahl, and D. R. Mathieson at the Mayo Clinic

(*Proc. Staff Meetings Mayo Clinic* **27,** *193 (1952)*). Alkaptonuria is a condition in which the urine contains alkaptons, substances that darken on standing or on addition of alkali. In spontaneous alkaptonuria the darkening is due to homogentisic acid and must be distinguished from that associated with phenol or benzene poisoning and melanuria.

Ochronosis is a metabolic condition in which alkapton bodies are deposited in certain tissues, most commonly bone, cartilage, ligaments, and skin. These are not exclusively human metabolic errors, for ochronosis has been described as affecting cattle, dogs, and horses. Alkaptonuria has been noted in rabbits. About 200 cases of alkaptonuria and 100 cases of ochronosis have been reported. Males are affected more frequently than females.

Spontaneous alkaptonuria is a hereditary, familial, and congenital anomaly of the metabolism of phenylalanine and tyrosine. It is usually inherited as a recessive Mendelian trait. Consanguinity favors its occurrence. In normal persons an intermediate product of the catabolism of tyrosine and phenylalanine appears to be homogentisic acid, which is ultimately metabolized to acetoacetate. In affected persons, however, the homogentisic acid is not oxidized normally and is excreted in the urine.

Homogentisic acid disappears when added to normal serum, but not when added to the serum of a patient with spontaneous alkaptonuria (G. Katsch and G. Stern, *Deutsch. Arch. f. Klin. Med.* **151,** *329 (1926)*). Homogentisic acid, phenylalanine, or tyrosine given to a patient with alkaptonuria results in correspondingly increased excretion of homogentisic acid. When these substances are given to a normal person, homogentisic acid is not recovered from the urine.

In most cases the condition is present at birth and continues throughout life. Patients with alkaptonuria usually exhibit no symptoms or secondary signs. In severe or long-standing cases alkaptonuric individuals may develop secondary ochronosis which may lead to crippling osteoarthritis. This is attributable to extensive degeneration of the joint cartilages from irritation caused by the foreign material, alkaptons, deposited therein. The clinical features may be summarized in the triad: dark urine, pigmentation, and arthritis.

The diagnosis is suggested early in life by the dark stains from urine on the diapers and linen. This stain is not removed by laundering and may be intensified by alkaline soaps. Later in life homogentisuria may be mistaken for glycosuria since alkaline copper solutions are reduced by homogentisic acid. However, homogentisic acid is not fermented by yeasts.

Grayish-blue or bluish-black deposits are particularly likely to be found in cartilaginous structures of the ear, nose, and larynx. The joints most often affected by ochronotic arthritis are those of the spinal column, shoulders, knee, and hips. Clinically the condition resembles rheumatoid arthritis, and roentgenographically it resembles osteoarthritis.

Ocular ochronosis may be seen as an accumulation of brown or gray pigment in the sclera midway between the margin of the cornea and the outer or inner canthus. It occurs here specifically because it attacks the insertion of the rectus tendon. Scleral ochronosis has been misdiagnosed as melanosarcoma with resultant enucleation. The eyelids may be pigmented, as well as the skin of the nose, cheeks, and axilla.

Homogentisic acid is excreted by the ceruminous and sudoriferous glands, thus resulting in dark ear wax and sweat stains.

Urinary changes may be helpful in diagnosis. Freshly voided urine is not unusual if it is acid; if alkaline it appears brown. On exposure to air it gradually darkens. Alkali speeds the change to brownish-black. A drop of alkalinized urine containing homogentisic acid will cause immediate blackening of exposed photographic paper or film (Fishberg test). No other substance excreted in the urine is known to cause this reaction. Alkalinization of the urine with

oxidation of homogentisic acid is followed by chemiluminescence, which may be observed in a dark room.

The most characteristic and distinguishing features of the 3 cases of alkaptonuria studied by this Mayo Clinic group are: (1) urinary staining of diapers and underclothing; (2) misdiagnosis of diabetes; (3) bluish discoloration of the cartilages of the ears and pigmentation of the sclerae; (4) confirmation of the presence of urinary homogentisic acid by means of Benedict's reagent (grade I atypical reaction) and Fishberg's test; (5) roentgenographic changes characteristic of ochronotic spondylitis (inflammation of the vertebrae).

Experimentally induced alkaptonuria can be produced by feeding L-tyrosine to normal subjects. With large doses of vitamin C, the excretion of homogentisic acid ceases. Although ascorbic acid appears to correct the experimentally induced alkaptonuria, it has little effect on the hereditary type. However, intramuscular injection of liver will free the urine of homogentisic acid for several hours (G. W. Thorn and K. Emerson *in* "Principles of Internal Medicine," edited by T. R. Harrison, *p. 708, Blakiston, Philadelphia (1950)*).

Treatment at present is not well defined. Nitrogen balance may be improved and homogentisic acid excretion reduced by administering a diet restricted in protein but high in carbohydrates and fat. Ascorbic acid in large quantities, 0.5 to 1.0 g. per day or more, may be tried. The possible beneficial effects of liver extract given daily for prolonged periods should be investigated.

MINERAL OIL DOSAGE AND VITAMIN A ABSORPTION

The ability of mineral oil to impede the absorption of vitamin A and of carotene from the human intestine, provided the correct time relationships of the administration of the oil and the nutrient are met, has been well established. The dosage relationships which have been less well studied are the subject of a recent investigation by F. Steigmann, H. Popper, H. Dyniewicz, and I. Maxwell (*Gastroenterology* **20,** *587 (1952)*).

In a series of control studies using diets containing 3000, 2500 and 1500 I.U. daily of vitamin A activity, most of which was present as carotene, these workers found that average vitamin A plasma levels tended to remain the same over three- to four-week periods in subjects receiving the 2500 I.U. dosage. The higher and lower intakes resulted in average increases or decreases respectively. Carotenoid levels did not fluctuate with the different regimens as markedly as did the vitamin levels. Because of the relative constancy obtained with the 2500 I.U. intake they studied the effects of various dose schedules of mineral oil on plasma vitamin A concentration in patients receiving this amount of vitamin.

Eighteen patients receiving 30 cc. of mineral oil mixed with the food of the noon meal developed an average decrease of 10 micrograms per cent (from 47 to 37 micrograms per cent) in the plasma vitamin A concentration over a four-week period. The same dosage administered at bedtime to 10 other patients during a similar period, however, produced no significant lowering. Two subjects given 10 cc. with each meal showed an average decrease in plasma values from 44 to 26 micrograms per cent, while 5 cc. doses given to 14 patients with each meal produced less striking changes, with an average fall of 8 micrograms per cent. Eight patients given 2.5 cc. with each meal showed no fall in plasma vitamin A, although this group differed from the others in having a markedly lower initial average plasma level (30 micrograms per cent) than any of the other groups. No change was observed in 2 patients given only 5 cc. of mineral oil with

the noon meal. In a study of 4 patients receiving 3000 I.U. of vitamin A, the administration of 30 cc. of oil mixed with the noon meal produced a fall from 42 to 31 micrograms per cent in a three-week period. On all schedules the plasma carotene concentration roughly paralleled that of vitamin A, but the authors believed the fluctuations were too marked to allow valid conclusions to be drawn.

The results reported in this investigation appear to indicate that 2.5 cc. of mineral oil mixed with each of the three meals represents an approximate threshold dosage below which no effect on vitamin A absorption is observable. This conclusion, however, is marred somewhat by the fact that the group of patients receiving this amount had lower initial levels than the groups receiving other regimens. Since all data are reported as averages, no analysis of individual patients is possible and no explanation of this discrepancy is offered by the authors.

Of perhaps even more significance in the evaluation of this report is the fact that no figures are given for the amounts of vitamin A activity contained in each meal. Therefore it cannot be determined whether the mineral oil ingested with only the noon meal was given simultaneously with the largest or smallest amount of dietary vitamin A (or carotene). Also, in view of the apparent potentiation of carotene activity by feeding it in margarine (*Nutrition Reviews* **9,** *331 (1951)*), it would be of some interest to know whether the carotene in the diets used was to any significant extent contained in margarine. If so, this might have influenced the results obtained, depending upon the time relationships involved.

Despite the defects mentioned, this paper represents a contribution to our rather crude knowledge of the absorption of vitamin A as it is influenced by the presence of poorly absorbed oils in the gastrointestinal tract. Further extension of these studies appears to be indicated since answers to some of the questions raised above might prove of value in the practical therapy of patients with hypervitaminosis A (*Nutrition Reviews* **9,** *183 (1952)*).

ASSESSMENT OF GROWTH AND DEVELOPMENT IN CHILDREN

Measurement of growing children has long been a standard part of the medical examination and of major concern in public health and preventive medical programs, especially those dealing with school children. It has been supposed, with considerable justification, that the rate of growth was an index of "nutrition" or at least of the "general condition" of children. Differences in growth rates between groups of children from varying socioeconomic and geographic groups have been repeatedly observed. Racial differences have not been so widely studied.

Interpretation of the significance of any measurement on any child depends upon the comparison between this measurement and those of a population of children of the same age, sex, and race. This comparison is necessarily a statistical one and depends for its success and usefulness upon sound statistical formulation of the standards used and upon intelligent interpretation. J. M. Tanner (*Arch. Dis. Childhood.* **27,** *10 (1952)*) has reviewed the available standards for growth from the statistical point of view. This critical analysis presents some of the many difficulties which are encountered in dealing with such standards.

Since the purpose of mensuration of children of school age is essentially a test of normality, adequate definition of "abnormal" is the first point discussed. An arbitrary definition is customarily used, and is designed to classify as "abnormal" a certain proportion of the population, say 10 per cent (*i.e.*, those children who fall above the 95th or below the 5th percentile for the

measurement in question). These limits may be varied at will to include a larger or smaller part of the group. Those children classified as "abnormal" by this means are not certainly abnormal, but are more likely to be so than a child whose measurement falls near the 50th percentile. In the final analysis determination of the normality of this child will depend on other means of examination.

While percentile distributions are relatively easily understood by those concerned with the health and growth of children, there are many advantages to the statistician in expressing the relationship between an individual and a population in terms of the mean and its standard deviation. The application of this method of analysis is appropriate only when the distribution of values is Gaussian or normal. While the distribution of height measurements is normal, that for weight is usually positively skewed and its variability is not accurately expressed in terms of standard deviations. In normal distributions percentiles may be expressed in terms of standard deviations.

Growth is a form of motion and may be characterized (1) by the *distance* achieved after a certain period of time, (2) by the *velocity* during a certain period of time, (3) in terms of acceleration or deceleration, and (4) in terms of the whole complex curve of growth from birth to maturity. Available standards are expressions of either *distance* or *velocity*. It is pointed out that velocity standards (in terms of growth increments per year) with their variability are not generally available since they must necessarily be derived from long-term longitudinal studies of fairly large groups of children. However, it is Tanner's opinion that velocity standards would permit earlier appreciation of the effects of pathogenic factors because time is necessary for the effects of changes in velocity to become manifest.

The majority of published standards for growth are distance standards. The averages for height, weight or other anthropometric measurement and a measure of their variability are given for certain ages. The presentation may be in the form of tables, or graphically as in the charts of H. C. Stuart and H. V. Meredith (*Am. J. Pub. Health* **36,** *1365 (1946)*), to give an American example. The accuracy of such standards is based on the care with which the original measurements were taken.

The use of distance standards for assessing velocity is criticized by Tanner (*loc. cit.*) because a relative change in position over a period of years, from the 95th to the 5th percentile in weight, for example, although giving a subjective impression of change in the velocity of growth, does not permit any critical assessment of the degree of the change in relation to the previously mentioned definition of "abnormality." It is also pointed out that at no time is the child in question "abnormal" according to the distance standards, although intuitively there is every reason to believe that such is the case.

The introduction of multiple measurement distance standards introduces many complicating factors for the statistician. The summation of "abnormals" found on the basis of each measurement alone will very likely be greater than 10 per cent, depending upon the extent to which the single measurements are correlated. This objection may be overcome by statistical means, however. One such means is the use of regression standards, *e.g.*, weight-for-height-for-age, the best known example being the Tables of B. T. Baldwin and T. D. Wood (*Mother and Child* **4,** *July Supplement (1923), American Child Health Association, New York*) which unfortunately do not include any measure of expected variability. A more recent example is the Wetzel Grid (N. C. Wetzel, *J. Pediat.* **22,** *82, 208, 329 (1944)*) which combines a weight-for-height regression distance standard with a distance-for-age standard. The weight-for-height regression distance standard is also used to measure velocity. This results in a method that is very aptly illustrative, but again

subject to the very valid criticism that the logarithm of the weight is probably not Gaussian in distribution. Wetzel's grid has practical usefulness despite the lack of evidence that the arbitrarily defined tolerance limit of ½ channel of deviation per 10 levels of development used bears a statistically proven relationship to the variability of changes in velocity.

The weight-for-height auxogram of V. Correnti (*Riv. di antropol.* **36,** *120 (1948/49)*, reprinted in *Yearbook of Physical Anthropology* **5,** *259 (1949)*) is considered by Tanner to have a much firmer biometric foundation. It consists of a height-on-weight regression plotted in the form of an ellipse containing a particular percentage of children of a certain age. Another distance standard used to measure velocity is A. W. Tuxford's index (*J. Hyg. (Cambridge)* **42,** *549 (1942)*) which has had wide use in England. This index is simply a weight/height ratio multiplied by a factor which corrects for age. An arbitrary limit of normal variation is used.

A method of assessing growth combining both distance and velocity standards is proposed by Tanner (*loc. cit.*). The results are expressed in terms of standard scores and their differences, which are compared with tables giving the corresponding percentiles. This proposed method has not yet been given a clinical trial. Its success will depend upon two factors; first, the success with which it indicates "abnormality" without the inclusion of too large a group of normals (R. H. Jones, *J. Roy. Statistical Soc.* **101,** *1 (1938)*); and second, the degree to which its final form is suitable for use by those persons usually untrained in statistics who are responsible for the care of the majority of children, the general practitioner and the school nurse.

Exact knowledge of the number of measurements necessary to assess with confidence the growth and general health of children, appropriate methods of analysis of these data, and criteria for their interpretation are not available. The rather widespread assumption that the normal child maintains the same relative position in a population during the years of linear growth has not been adequately documented. Even the criteria of "normal" vary. Available standards for growth must be used and interpreted with appreciation of these areas of uncertainty.

IODINE-TAGGED ALBUMIN IN CIRRHOTIC PATIENTS

Although some doubts have been cast on the theory that a decrease in serum protein concentration, particularly albumin, is the principal cause of nutritional edema, many investigators have demonstrated a correlation between the presence of ascites and a lowered serum albumin level in cirrhotic patients (*Nutrition Reviews* **4,** *317 (1946)*; **5,** *31 (1947)*; **6,** *210 (1948)*; **8,** *51 (1950)*).

The liver is the main, if not the only, site of serum albumin synthesis and the circulating albumin seems to be a reflection of the body's total store of labile albumin. The lability of protein stores was demonstrated in early tracer studies. A novel approach to the problem of studying the lability of serum albumin in normal and cirrhotic individuals was recently reported by M. P. Tyor and D. Cayer (*South. Med. J.* **45,** *141 (1952)*). These workers "tagged" human serum albumin with radioactive iodine (I^{131}) and injected the "tagged" protein into 11 control patients without liver disease, 7 cirrhotic patients without ascites, and 5 patients with cirrhosis and ascites.

The degree of radioactivity of the serum was measured twenty-four, forty-eight, and seventy-two hours after injection of the "tagged" albumin. The plasma volume was determined from the degree of radioactivity

ten to fifteen minutes after injection. Serum albumin concentrations were determined by a chemical method (Kjeldahl). The total circulating albumin was calculated from the plasma volume and serum albumin level.

During the first twenty-four hours after administration of "tagged" albumin, the rate of its disappearance from the serum was the same in the 3 groups of patients. At forty-eight to seventy-two hours, however, the patients with cirrhosis and ascites had a markedly reduced rate of disappearance of the tagged albumin from the serum, an indication of an impairment in the rate of albumin metabolism in such patients. Furthermore, the patients with cirrhosis and ascites exhibited a significant reduction in the total circulating albumin and serum albumin concentration. Serum albumin concentrations were reduced, although to a lesser extent than in those with ascites, in the patients with nonascitic cirrhosis, but these patients had essentially normal total circulating albumin.

The data are consistent with the concept that in severe portal cirrhosis there is probably a marked interference with albumin synthesis. Although Tyor and Cayer believe that the decline in albumin synthesis is of prime importance in the pathogenesis of edema, they consider that factors such as electrolyte balance, portal hypertension, and possible endocrine disturbances are contributory.

INFLUENCE OF TOCOPHEROL INTAKE ON VITAMIN A CONTENT OF MILK

Recent work has indicated that there is a correlation between the tocopherol and provitamin A (carotene) content of cow's milk (*Nutrition Reviews* **9,** *118 (1951)*).

A recent report by W. A. McGillivray confirms this point of view and emphasizes further that pastures may become sufficiently low in tocopherols in the summer months to influence adversely the utilization of the carotene and thus decrease the total vitamin A potency of milk (*J. Dairy Res.* **19,** *119 (1952)*). This study, conducted at the University of New Zealand Agricultural College, indicated that pastures of rye grass and clover contained sufficient carotene to provide an average daily intake of 1.5 g. per cow daily. This level of intake is three to four times the amount needed to maintain the maximum vitamin A potency of milk of about 2000 I.U. per pound. In spite of the high-carotene pasturage, the cows were observed to produce milk with a vitamin A potency of about 1200 I.U. per pound in the summer months contrasted with the expected vitamin A potency of about 2000 I.U. units per pound in the winter and spring months.

To determine if the lowered vitamin A potency of the summer milk could be related to a decline in tocopherol intake, a study was conducted using 3 pairs of monozygotic twin cows. These animals were grazed on a rye grass-clover pasture and when the vitamin A potency of the milk had declined to about 1200 I.U. per pound in midsummer, one member of each pair of twins received 1 g. of alpha-tocopherol daily for fourteen days. In those receiving the tocopherol the vitamin A potency of the milk started to rise within two days and reached the maximum value of about 2000 I.U. per pound in eight days, while the control cows not receiving the supplement continued to produce milk with a vitamin A potency in the neighborhood of 1000 to 1200 I.U. per pound.

In a subsequent twelve-day experimental period it was established that raising the tocopherol supplement had no further influence on the vitamin A potency of the milk and that a combined supplement of

tocopherol (3 g. per cow per day) and beta-carotene (300 mg. of a 2 per cent carotene concentrate daily per cow), likewise was no more effective than the supplement of tocopherol alone.

During the course of these studies the pastures grazed had an average carotene content of 374 micrograms per gram. From this it was calculated that the cows were ingesting about eight times the amount of carotene needed to produce milk of maximum vitamin A potency. Tocopherol supplements nevertheless were needed to attain maximum vitamin A potency during the summer months.

In addition to confirming the earlier observation (*Nutrition Reviews, loc. cit.*) that the tocopherol and carotene content of milk are directly correlated, this study further revealed a highly significant correlation between the total vitamin A potency and tocopherol content of milk.

There was no mention of any adverse effects that could be ascribed to insufficient tocopherol intake other than the decline in vitamin A potency of the milk. That dairy cows under average practices and good grazing should require supplementary tocopherol is a new concept. It has generally been assumed (*Nutrition Reviews* **9,** *76 (1951)*) that ordinary rations, and particularly fresh forage, would supply liberal tocopherol intakes for cattle.

A vitamin E deficiency in young calves was recently described which occurred on a ration that was assumed to contain sufficient tocopherols to afford protection (K. L. Blaxter, P. S. Watts, and W. A. Wood, *Brit. J. Nutrition* **6,** *125 (1952)*). The ration used (a reconstituted milk made up of skim milk powder, lard, glucose, and minerals) was fortified with cod liver oil as a source of vitamins A and D. These workers concluded that 15 to 18 ml. of cod liver oil per calf daily rendered ineffective the protective action of a daily supplement of 50 mg. of DL-alpha-tocopherol acetate (Blaxter and Wood, *Ibid.* **6,** *144 (1952)*).

With regard to the results reported by McGillivray, it is possible that late summer forages may not only decline in tocopherols, but may also accumulate substances that oxidatively destroy carotene and tocopherols. No data were reported in the New Zealand study regarding the tocopherol content of the rye grass-clover pasture on which the cows were grazed.

MODE OF ACTION OF ANTIBIOTICS IN POULTRY NUTRITION

The mode of action of antibiotics as growth stimulants in animals receiving presumably adequate diets has not been established. Many have speculated that the effects of the antibiotics can be explained by their selective action on intestinal microorganisms. These microorganisms in competition with the host for essential dietary factors, for example, could jeopardize the nutritional status of the host on presumably adequate diets. Removal of such organisms by the feeding of antibiotics would then improve the nutrients available to the host. This explanation appears to fit the recent observations that the antibiotics improve the growth of rats fed diets marginal in some of the well known B-vitamins, namely, thiamine, riboflavin, pyridoxine, and pantothenic acid (*Nutrition Reviews* **10,** *107 (1952)*; H. E. Sauberlich, *J. Nutrition* **46,** *99 (1952)*).

New evidence for the mode of action of antibiotics is offered by some British workers at the University of Reading, and the Glaxo Laboratory Ltd. (M. E. Coates *et al.*, *J. Sci. Food Agr.* **3,** *43 (1952)*). These workers suggest that at least one action of antibiotics in chicks fed normal rations is to eliminate an infectious condition that is readily transmissible.

Day-old chicks fed a commercial ration, presumably adequate in known dietary essentials, gave growth responses to penicillin at the University of Reading laboratory, but the penicillin failed to stimulate growth when added to the ration at the Glaxo Laboratory. In the initial experiment Rhode Island Red and Light Sussex crosses obtained from different sources were used in the two laboratories.

The growth obtained without the antibiotic at the Reading laboratory was inferior (143 g. body weight in three weeks) to that obtained without antibiotic at the Glaxo laboratory (220 g. body weight in three weeks), and the penicillin supplement at the former location merely restored growth to the presumably normal level of about 220 g. body weight at three weeks of age. The ration used was made up of a variety of vegetable proteins and contained 5 per cent of fish meal, 5 per cent of dried whey, and 2.5 per cent of whole meat meal as animal protein sources.

In order to investigate the difference in behavior of chicks grown in the two laboratories a joint study was conducted. Eighty chicks from a single hatch, and a single batch of ration were divided between the two laboratories. In each laboratory the chicks were divided into 4 treatment groups as follows: Groups 1 and 3 received the ration without supplement; Groups 2 and 4 received a penicillin supplement (40 mg. of procaine penicillin per kilogram of ration); Groups 1 and 2 were kept on a raised wire floor, whereas Groups 3 and 4 had access to a sawdust litter which was changed once a week. The initial observation was confirmed in that the chicks at the Reading laboratory again required the penicillin supplement in order to attain the normal growth observed at the Glaxo laboratories where penicillin had no effect.

Since ration and genetic effects were presumably well controlled in this study, it appeared that some other environmental factor was responsible for the poor growth obtained without penicillin at the Reading laboratory. At this laboratory chicks had been kept for a number of years on the same premises whereas chicks had not been kept previously at the Glaxo laboratory.

A third experiment was conducted in three locations, the two locations used earlier and another location (new building) at Reading where chicks had not been kept previously. The ration and genetic background of the chicks were again controlled. Only the chicks at the old location at Reading where chicks had previously been kept responded to penicillin. Those in the new location at Reading where no chicks had been kept previously and those at the Glaxo laboratory made normal gains without penicillin supplement.

After a two-week period chicks from all three locations were placed in the same pen to determine whether or not the chicks grown in locations where penicillin was needed for normal growth might carry "infections" that would be transmitted to the chicks that had no requirement for the antibiotic. Under these conditions the chicks previously shown not to require penicillin now needed the antibiotic in order to continue their normal growth rate.

Several explanations for these results were offered by the authors. The most plausible seemed to be the presence of an infectious agent on premises where chicks had been grown previously. The only abnormality noted was a growth depression under these conditions that could be corrected with penicillin.

In another paper W. F. J. Cuthbertson (*J. Sci. Food Agr.* **3,** *49 (1952)*) indicated that the above explanation of the beneficial effects of dietary penicillin could not explain the superior growth noted in some further studies in which chicks and rats were fed all-vegetable rations supplemented with penicillin.

Unfortunately, in the above studies where the mode of action of penicillin is explained as overcoming an infection, no attention

was given to the possibility of an anthelmintic action of the antibiotic. A. C. Todd has reported (*Poultry Sci.* **30,** *763* (*1951*)) that penicillin, fed at the rate of 6.8 mg. per pound of feed, inhibited the development of *Ascaridia galli* in the intestine of chickens. Fifteen mg. of antibiotic per pound of feed produced significant reductions in the number of worms developing from controlled exposure. Unfortunately, these tests were likewise not conducted in such a manner that the superior weight gains of antibiotic-treated chicks could be identified as due to the anthelmintic action of the antibiotic.

The British workers made reference to studies by R. L. Davis and G. M. Briggs (*Poultry Sci.* **30,** *767* (*1951*)) who likewise failed to obtain uniform responses to penicillin when the chicks were housed in new quarters. Furthermore, the chicks housed in the new quarters consistently made better weight gains in the absence of antibiotics than could be obtained when the chicks were housed in old quarters.

These results are highly suggestive that much of the benefit derived from antibiotics in poultry nutrition may involve the control of transmissible agents which accumulate in quarters where chicks are kept year after year.

PYRIDOXINE AND SYNTHESIS OF PYRIDINE NUCLEOTIDES

Evidence demonstrating that pyridoxine plays a role in the transformation of tryptophan into niacin has been discussed previously (*Nutrition Reviews* **9,** *272* (*1951*)). Pyridoxine deficiency in rats is followed by the appearance in the urine of an abnormal product of tryptophan catabolism, xanthurenic acid (S. Lepkovsky and E. Nielsen, *J. Biol. Chem.* **144,** *135* (*1942*)). The concentration in the urine of some of the normal derivatives of niacin is decreased (C.-T. Ling, D. M. Hegsted, and F. J. Stare, *Ibid.* **174,** *803* (*1948*)). Pyridoxine deficiency inhibits the excretion of niacin itself and of quinolinic acid after the administration of either tryptophan or kynurenine (L. M. Henderson, I. M. Weinstock, and G. B. Ramasarma, *Ibid.* **189,** *19* (*1951*)).

On the other hand, a number of workers (H. Spector, *J. Biol. Chem.* **173,** *659* (*1948*); E. Geiger, E. B. Hagerty, and H. D. Gatchell, *Arch Biochem.* **23,** *315* (*1949*); and M. Heimberg, F. Rosen, I. G. Leder, and W. A. Perlzweig, *Ibid.* **28,** *225* (*1950*)) have found themselves unable to demonstrate that pyridoxine deficiency exerted a specific effect on tryptophan metabolism. For example, Spector found no decrease in the rate of excretion of niacin or N-methylnicotinamide after tryptophan administration in pyridoxine deficiency. J. N. Williams, P. Feigelson, S. S. Shahinian, and C. A. Elvehjem (*Proc. Soc. Exp. Biol. Med.* **76,** *441* (*1951*)) found that rats made pyridoxine-deficient could still convert tryptophan to liver pyridine nucleotides, which contain the niacin structure. It appeared possible that this continued conversion was due to the fact that the simple absence of pyridoxine from the diet, however prolonged, does not sufficiently deplete the tissues of pyridoxine. High dietary levels of tryptophan, such as are used in this type of experiment, tend to facilitate niacin synthesis, even when concentration of pyridoxine is far from optimal.

In order to study the conversion of tryptophan into liver pyridine nucleotides under more severe conditions of pyridoxine deficiency, J. P. Kring, K. Ebisuzaki, J. N. Williams, and C. A. Elvehjem (*J. Biol. Chem.* **195,** *591* (*1952*)) employed the addition of desoxypyridoxine to the experimental diet. The mode of action of this antivitamin (*Nutrition Reviews* **8,** *28* (*1950*)) appears to be through its conversion into an analogue of pyridoxine containing coenzymes, presumably desoxypyridoxine phosphate, after which it competes with

pyridoxal phosphate for the apoenzymes. In particular, it seems to interfere with the combination of pyridoxal phosphate with the specific protein to yield tyrosine decarboxylase. It may act similarly in the case of the pyridoxine-containing enzymes active in the conversion of tryptophan to niacin.

Kring and her collaborators (*loc. cit.*) first depleted weanling rats of liver pyridine nucleotide by feeding them for a period of nineteen days a protein-free diet and then depleted them of pyridoxine and of niacin by feeding them for two to three weeks a basal diet free of these vitamins. Sulfasuxidine was added to prevent intestinal synthesis of the vitamins. Some of the animals also received desoxypyridoxine during this depletion period. Supplements of desoxypyridoxine, niacin, tryptophan, pyridoxine, or combinations of two or three of these substances were then added to the basal diet so that a total of 16 experimental groups were constituted. At the end of the experimental period of one week, the animals were killed and liver pyridine nucleotides were determined.

It was found that simple pyridoxine deficiency decreased the nucleotide content of the liver by about 40 per cent. Supplementation with either tryptophan or niacin brought the level back to normal. Giving both supplements had an additive effect on the nucleotide level. Thus, it was confirmed that simple pyridoxine deficiency did not appreciably decrease the ability of the organism to convert tryptophan into niacin. In the case of the animals receiving desoxypyridoxine, it was found that, while the administration of niacin still had an effect of the same order on liver pyridine nucleotide as in simple pyridoxine deficiency, that of tryptophan supplementation was about 50 per cent smaller. The effect of administering both supplements in the presence of desoxypyridoxine was again the sum of the effects of administering each supplement in the presence of the antivitamin. It appears, therefore, that desoxypyridoxine does, in fact, decrease the conversion of tryptophan to pyridine nucleotide although it does not block it altogether. It seems clear that the reactions involved in the synthesis of ribose or those concerned with the formation of the niacin riboside are probably not affected by pyridoxine.

These experiments can be taken as additional proof that pyridoxine plays a role in tryptophan metabolism. They give no additional clue, however, to the mechanism of this particular action of the vitamin.

NERVOUS SYSTEM AND ANIMAL BEHAVIOR IN B-COMPLEX DEFICIENCIES: CONDITIONED RESPONSES

Deficiency of several nutrients results in an impairment of functions mediated by the cerebral cortex. The ability to learn is one such function amenable to quantitative study. Two modes of learning may be distinguished (G. Allport): efferent learning, involving *alteration in the response* to a given set of stimuli, and afferent learning, consisting in the *extension of the range of effective stimuli*. Learning to run a maze (*Nutrition Reviews* **10,** *235 (1952)*) and the acquisition of a conditioned response may serve to illustrate the two kinds of learning. Conditioned responses seem to be highly sensitive indicators of nutritional impairment of the central nervous system. Strangely enough, nutritionists have neglected this technic, placing an almost exclusive reliance on maze learning.

It has been noted (*Nutrition Reviews, loc. cit.*) that water mazes are preferable to dryland mazes in studying the effects of vitamin B-complex deficiencies on learning capacity. However, swimming involves large

musculature and when muscular weakness of the extremities is present, it alone could result in an inferior maze performance without an alteration in the central component of performance (*i.e.*, in the learning ability).

The effort to eliminate the interference of variable motivational factors and to reduce the importance of muscular weakness of the extremities led W. C. Biel and D. C. Wickens (*J. Comp. Psychol.* **32,** *329 (1941)*) to apply the conditioned response technic. A puff of air, applied to the cornea of the right eye, served as the normal (unconditioned, direct) stimulus and resulted in closure of the eyelid. The appearance of light was used as the conditioned stimulus, evoking by itself, after a series of exposures to both light and puff of air, the eyelid response (a conditioned reflex). Establishment of a conditioned reflex is a simple, basic tool for the evaluation of the animal's ability to develop new stimulus-response relationships. In certain nutritional deficiencies this approach is a more effective test of animal intelligence than the learning of a maze.

When the speed of acquisition of the conditioned responses was determined by Biel and Wickens in 15 white rats, deprived of most of the dietary thiamine from one week of age and compared with 15 normal littermates at 50 to 70 days of age, the control animals made a significantly higher mean score (33.7 versus 22.3 per cent of positive responses for a given testing period). It should be noted that the deficiency did not affect the sensitivity of the animals to the unconditioned stimulus; the reaction time to air puff alone was 56.7 and 57.5 milliseconds for the control and the deficient animals, respectively.

Biel and Wickens used the formation of a simple conditioned motor response as a criterion of the efficiency of the cerebral cortex. It may be expected, on theoretic grounds, that more complex conditioned responses would provide a more sensitive yardstick for the detection of disturbances of cortical function. Such a criterion is provided by the formation, maintenance, and disintegration of so-called "differential inhibition" (*see* J. Konorski, "Conditioned Reflexes and Neuron Organization," *p. 149 ff., Cambridge University Press (1948)*). Two different tones, two speeds of the metronome, two geometric figures, etc.—one always followed (reinforced), the other not followed by food—may be used for establishing conditioned inhibition.

Perhaps a short historical note on the early use of this method in nutritional research may be of interest. The effects of prolonged caloric deprivation on the functional status of the cerebral cortex were studied by the technic of conditioned reflexes by I. S. Rozental (*Arkh. biol. nauk* **21,** *151 (1922)* and J. P. Frolov (*Ibid.* **21,** *159 (1922)*). The problem was thrust at the two physiologists working in the laboratory of I. P. Pavlov at St. Petersburg (today's Leningrad) when the food supplies became greatly reduced in 1918/1919. Incidental observations were made on 13 dogs. Two animals, which during this period of observation lost more than 40 per cent of their initial body weight and finally died, were studied in detail. The loss of differential inhibition was an early symptom of the deterioration of the functional capacity of the central nervous system. In time, well established conditioned responses also decreased in intensity. It was noted that the sequence in which the cortical functions disappeared under conditions of prolonged starvation was exactly opposite to the order in which they were established. The most recently acquired and the most complex types of conditioned responses were lost first while the unconditioned reflexes lasted until the time of death.

W. H. Gantt, once Pavlov's research associate ("Experimental Basis for Neurotic Behavior," *p. 2, Hoeber, New York (1944)*), reported a rapid deterioration of differential inhibition in a dog on a diet

deficient in pyridoxine (vitamin B_6) and pantothenic acid. As early as the third day the dog began to show impaired differentiation and the impairment became worse as time went on. When the animal was placed on an adequate diet, the differentiation improved rapidly and returned to the predeficiency level of perfection.

A study on the effect of vitamin B-complex on higher nervous activity in dogs, carried out at the Pavlovian Laboratory of Johns Hopkins University, was reported by W. H. Gantt and M. Wintrobe (*Fed. Proc.* **4,** *22 (1945)*). It is of interest to note that the conditioned reflexes were more sensitive criteria of altered nervous function than any other criterion available. At a time when the animals failed to differentiate between the stimuli there were no observable changes in behavior, in unconditioned reflexes and in blood composition. It is unfortunate that the study has not been reported in full detail, both as to the dietary conditions and the process of deterioration of the conditioned differential inhibition.

The role of thiamine in cortical activity of the dog has been investigated by the technic of conditioned reflexes by L. O. Zevald. The studies were published in the *Trudy (Works) of the Pavlov Physiological Laboratories* and only recently became accessible to the reviewer. Zevald, true to Pavlov's preferred procedure, used the secretion of saliva (not a motor response) as the indicator of a conditioned response (*Ibid.* **16,** *253 (1949)*). Two dogs were used as experimental subjects. The conditioned responses remained at a relatively stable plateau during twenty-three days of maintenance on the unautoclaved experimental diet. The average strength of the salivary response to the bell, electric light, and the metronome (as read from the author's graphs) was about 50, 41, and 49 units, respectively. The nonreinforced auditory stimulus (metronome at 60 beats per minute) was followed by a weak secretory response (20 units).

A decrement in the salivary response to the positive (reinforced) stimulus and a prolongation of the latent period began to be noticeable after three days of feeding the autoclaved food. Within a week the responses became highly variable and the ability to differentiate between the two metronome speeds deteriorated. The intensity of secretory response to positive stimuli became weaker, with values of 25, 13 and 37 units. The animal developed anorexia, began to lose weight, reduced its spontaneous activity and spent much of the time lying down in the kennel. Later it became unable to jump up at the experimental table or even to stand up on the table, and readily lost balance even when sitting.

After three and one-half months the animal lost 25 per cent of the initial body weight, its gait became ataxic, and conditioned secretory activity was deeply depressed, with values of 15, 4 and 6 units. The nonreinforced (negative) stimulus was followed by 8 units of secretion, *i.e.*, the differentiation was abolished. Daily injections of massive doses of thiamine (50 mg.) given for a week raised the secretory responses to 40, 15, and 27 units. The clinical symptoms of athiaminosis (vomiting, refusal of food, and motor disturbances) disappeared on the second day of supplementation. Even after a maintenance on good diet for three months the conditioned responses did not regain the original intensity, but the differentiation was fairly well established (salivary response of 17 units, versus 31 units for the reinforced stimulus).

In the second dog a fistula was used for placing food in the animal's stomach when it refused the food used for alimentary reinforcement of the conditioned responses. Also, the food not eaten by the anorectic animal during the regular feeding period was introduced into the stomach in this way. As one would expect, this procedure did not prevent gastrointestinal disturbances (diarrhea, vomiting) and loss of weight. The

breakdown of the conditioned responses paralleled the development seen in the first dog, indicating a disturbed function of the cerebral cortex.

Another paper (L. O. Zevald, *Trudy* **15,** *259 (1949)*) was concerned with the possibility of establishing new conditioned responses in dogs in a state of acute thiamine deficiency. This was demonstrated in 2 out of 3 experimental animals used in the study. However, the magnitude of the newly established responses was below the level characteristic of stimuli of the same strength and conditioned during the period of normal diet, preceding the period of deficiency.

In several respects Zevald's reports are incomplete. For instance, inadequate data are given on changes in the body weight during the period of deficiency and of refeeding. More importantly, no figures are given on the water intake of the animals—a factor which by itself may account for at least a part of the differences in the amount of salivary excretion. These experiments deserve to be repeated with a better control of the state of hydration and a more systematic variation of the levels of thiamine supplementation.

The technic of conditioned responses as means for a physiologic study of an important aspect of "animal intelligence" should be used more extensively in the evaluation of dietary factors involved in the maintenance of a normally functioning central nervous system and, more specifically, of the brain cortex.

STRAIN VARIATION AND CARIES SUSCEPTIBILITY

In dental caries research, frequent discussions arise among investigators as to why such different caries susceptibilities are observed under seemingly identical or highly similar experimental environments. One phenomenon which undoubtedly is involved in these variations has been investigated by J. O. Holmes (*J. Nutrition* **46,** *323 (1952)*), who has reported a series of studies on the caries susceptibility of three strains of laboratory rats (*Mus norvegicus*) fed finely powdered, purified rations.

The first strain of rats was an albino variant which was designated as MW; the original animals of this strain were purchased from The Wistar Institute in 1934 and inbred until 1945 when several Sprague-Dawley males were introduced into the colony. The second strain was a heterozygous black rat (MB) purchased in 1943 from Carworth Farms. The original rats of the third strain (C) were purchased in 1947 from the Connecticut Agricultural Experiment Station and were inbred descendants of a stock of unknown origin into which a few Wistar Institute males had been introduced in 1912 and again in 1918.

The young rats were fed the experimental ration beginning at 21 to 30 days of age and were kept in individual wire-mesh cages on screen floors. Distilled water and rations were provided ad libitum. The experimental rations were composed of vitamin-free casein, 15 to 25 per cent; salt mixture, 4 per cent; corn oil, 4 per cent; liver fraction L, 4 per cent; and sucrose or glucose, 63 to 73 per cent. Adequate amounts of the water- and fat-soluble vitamins were fed either in liquid form daily or mixed into the ration. The rats in strain MW and MB were maintained on the experimental regimen for ninety-eight days; the rats from strain C were maintained for periods of ninety-eight, seventy-seven, or sixty days.

The results of these studies indicated that there was a striking difference in susceptibility among the three strains when maintained under identical environmental circumstances. The 106 representatives of the MW strain were the most resistant, with

94 per cent of them developing carious lesions. The 63 members of the MB strain were intermediate with respect to caries incidence, with 98 per cent of them having some tooth decay at the end of the ninety-eight-day experimental period. The C strain was most susceptible, with none of the 29 members tested being immune. Further indications of the different caries susceptibilities of the three strains were provided in the average number of carious teeth, the average number of carious lesions, the average number of lesions which had progressed to large cavities, and the average caries score. For example, the average numbers of carious lesions per rat for the three strains, MW, MB, and C, were 8.7 ± 5.8, 14.1 ± 8.1, and 13.9 ± 6.6, respectively. The average caries scores per rat were 14.7 ± 13.3, 26.6 ± 20.1, and 37.8 ± 23.8, respectively. In the 91 and 112 representatives of strain C which were maintained on the experimental regimen for seventy-seven and sixty days, respectively, even higher average numbers of carious lesions per rat and higher average caries scores were observed than in the smaller group maintained under the same circumstances for ninety-eight days.

J. Haldi and W. Wynn (*J. Nutrition* **46,** *425* (*1952*)) have studied the caries susceptibility of a strain of rats inbred in their laboratories for a period of over seventeen years. The original rats used to develop this colony had been obtained from the Wistar strain. In the first experiment, 24 groups of litter mates of 4 animals each were selected at weaning from parents that had been maintained throughout life on a stock diet composed of a commercial laboratory chow. One rat from each of the 24 quartets was maintained after weaning on one of the four following diets: high sucrose, high protein, high fat, and a stock diet of laboratory chow. Thus there were 24 rats in each group.

The high sucrose diet contained 64 per cent sucrose, 20 per cent casein, 8 per cent fat, 4 per cent of a yeast and liver extract, and 4 per cent of a salt mixture. The high protein diet contained an extra 42 per cent casein in place of a comparable amount of sucrose. The high fat diet contained an additional 5 per cent of casein and an additional 35 per cent of fat, both replacing equal weights of sucrose. At the end of one hundred and fifty days on the experimental diets, the rats were killed and the teeth were examined under the microscope for carious lesions.

Of the 24 rats on the high sucrose diet, only 12 had carious lesions; in these 12 rats there was a total of 24 lesions, all of which were relatively small. Two rats in the high protein group had a total of 7 small lesions. Four rats in the high fat group had a total of 8 small lesions. Four rats in the stock ration group had a total of 6 small lesions. In other words, the incidence of caries was remarkably low in this strain of rats under the circumstances of this experiment.

In a second investigation, these workers selected from their stock colony litters which could provide 4 males and 4 females. These young rats were divided into 4 groups in such a way that one male and one female from each litter could be maintained throughout the rest of their experimental lives on each of the four diets described above. At the age of 100 days, the pairs were mated. After weaning, the offspring of these animals were maintained for a one hundred and fifty-day period on the same diet as their parents had been fed. At the end of this time, they were killed and their teeth were evaluated for caries. Again the caries incidence in all 4 groups was very low, with no increase in caries noted as a result of the feeding of the purified rations throughout gestation and lactation.

The results throughout both of the experiments of Haldi and Wynn indicate that the summation of all systemic and environmental circumstances had produced a very low incidence of tooth decay in this

population of rats. The final caries incidence of these rats was much lower than the most resistant of the three strains used by Holmes. From the data presented in these papers, the two obvious differences between the studies from the two laboratories were the strains of rats and the diets used. Possibly other less tangible variants were present, such as housing conditions and variations in drinking water, which may have contributed to the differences in caries incidence. Attention should be drawn to the fact that the high carbohydrate diets used in the two laboratories were quite similar on the basis of percentage composition, but did differ in a number of small ways. However, the fact that the percentage distribution of the major components was similar is not sufficient evidence to conclude that the caries-producing properties of the diets were identical. Simple consideration of the diets on the basis of percentage composition is insufficient, for it overlooks the very important consideration of when and how the individual components were grown or produced and treated thereafter. More and more in the dental caries field, it is becoming apparent that there may be minute differences which play highly important roles. As yet, it is impossible to characterize exactly what these differences are or how they operate.

Haldi and Wynn drew attention to their observation that feeding the purified diet during tooth development did not increase the susceptibility to tooth decay of these animals in the second experiment. This was in contradistinction to the observations of R. F. Sognnaes (*see Nutrition Reviews* **6,** *196 (1948)*) that this procedure resulted in a higher susceptibility to caries among the animals in the second generation. On the surface, there would appear to be at least two possibilities to explain the difference in results. First, the strain of rats used by Haldi and Wynn may have been sufficiently caries-resistant that the purified diet was unable to overcome the genetic influence. Second, the purified diet used by Haldi and Wynn may have been sufficiently different in some detail from that used by Sognnaes to have produced a difference in the caries susceptibility of the rats comparable to that produced by the natural diet.

These studies point out very clearly certain pathways along which much further experimentation is required in the dental field. Why do strains of experimental animals differ so markedly in their caries susceptibility under standarized conditions? What are the differences between diets fed during tooth development which produce different rates of caries initiation and development?

METABOLIC FATE OF ORALLY ADMINISTERED CHOLINE

Choline has been widely used in the treatment of diseases of the liver in an effort to take advantage of its activity as a lipotropic agent. However, relatively little information is available concerning the metabolic fate of the administered choline. In certain patients with portal cirrhosis it has been found that choline administered early in the course of treatment increases the rate of synthesis of phospholipids (*Nutrition Reviews* **8,** *284 (1950)*). This has led to the view that there is a deficient supply of choline or its precursors in these patients and that a portion of the supplements of choline provided is utilized in the synthesis of phospholipids. It is not known how disorders of the liver may otherwise influence the utilization of choline.

Additional information concerning the metabolic fate of choline in normal individuals and in patients with diseases of the liver has been provided in a number of re-

cent publications of investigators at the Hektoen Institute for Medical Research in Chicago. J. de la Huerga and H. Popper (*J. Clin. Invest.* **30,** *463 (1951)*) found that little choline was excreted in the urine of normal subjects on usual diets or after the ingestion of 2 to 8 g. of choline. However, about two-thirds of the orally administered choline could be accounted for by the urinary excretion of trimethylamine and trimethylamine oxide, metabolic products of choline. Patients with diseases of the liver excreted substantially less trimethylamine after ingestion of supplements of choline. There also was a delay in excretion of the metabolite.

Evidence is presented that trimethylamine is derived from bacterial metabolism of choline within the intestinal tract. Administration of the antimicrobial agents aureomycin and sulfathalidine to reduce the number of intestinal organisms caused a substantial decrease in the quantity of trimethylamine excreted. Moreover, cultures of fecal bacteria converted choline to trimethylamine.

Further evidence of the intestinal origin of trimethylamine is given in a second paper (J. de la Huerga, H. Popper, and F. Steigmann, *J. Lab. Clin. Med.* **38,** *904 (1951)*). In these experiments choline chloride in quantities of 1.15 to 2.30 g. was injected intravenously into normal subjects and patients with hepatic diseases. There was no significant increase in the urinary excretion of trimethylamine when choline was administered by this route. This observation gives additional support to the view that trimethylamine originates in the intestine and that body tissues are not importantly involved in effecting the conversion from choline. It was observed also that urinary loss of choline given intravenously was much greater in patients with acute hepatitis than in normal subjects or in patients with cirrhosis.

In a more recent publication results of experiments are described in which the metabolism of choline has been investigated in rats with and without induced hepatic injury (H. Popper, J. de la Huerga, and D. Koch-Weser, *J. Lab. Clin. Med.* **39,** *725 (1952)*). Normal rats were found to excrete trimethylamine in urine after introduction of choline through a stomach tube, the amount excreted accounting for about 40 per cent of the choline nitrogen. None of the choline administered could be recovered from the feces. As in human subjects, parenteral administration of choline was not followed by an increase in urinary trimethylamine. The urinary loss of choline was less than 1 per cent when the lipotropic agent was given orally and about 8 per cent when injected.

Intestinal bacteria were obtained for culture from selected sites in the stomach, small intestine and large intestine. Only from an area in the small intestine just below the entry of the common duct into the duodenum did organisms fail to grow. All other cultures grew and were effective in transforming large quantities of choline to trimethylamine.

When choline was administered in corn oil or in Tween 60 there was a reduction of about one-half in the urinary trimethylamine excreted. Similarly, giving choline as lecithin resulted in less urinary excretion of the metabolite. Neither choline nor trimethylamine was found in the feces of animals receiving choline with these other substances. Administration of atropine and prostigmine, drugs which influence intestinal motility and absorption, failed to effect the excretion of trimethylamine following oral choline administration.

A variety of procedures were utilized to induce hepatic damage in rats and the metabolism of choline was studied in these animals. Acute hepatic injury induced by carbon tetrachloride, bromobenzene, or ethionine failed to influence the production and urinary excretion of trimethylamine from orally administered choline. Chronic liver damage produced by carbon tetrachloride,

bromobenzene, ethionine, or butter yellow (paradimethylaminoazobenzene) was associated with significant reduction in urinary excretion of trimethylamine following ingestion of choline. In general this reduction was greater in animals with most marked liver cell damage. Choline-deficient animals with fatty infiltration of the liver but without liver cell damage showed only a slight decrease in excretion of trimethylamine. Dietary liver damage produced with a high-fat, low-protein diet was associated with a marked decrease in quantity of the metabolite excreted.

The observations of these authors indicate that trimethylamine is the chief metabolic product of ingested choline. No evidence has been found of other metabolic products. In cultures of intestinal bacteria all of the choline added to the medium can be accounted for as trimethylamine or choline. It is presumed that the choline which is not converted in the gastrointestinal tract is absorbed as such since choline has not been found in feces. It is also indicated that the rat absorbs a proportionally greater quantity than does the human subject since 40 per cent and 66 per cent of ingested choline is accounted for by the trimethylamine excreted by the rat and man respectively.

Trimethylamine is also rapidly absorbed by the intestine. None has been demonstrated in the feces of experimental animals or man. It is unlikely that this compound has any effect as a lipotropic agent (E. H. Mawson and A. D. Welch, *Biochem. J.* **30,** *417 (1936)*).

Both in patients with cirrhosis and in rats with experimental liver damage it has been shown that there is decreased conversion to trimethylamine and decreased excretion of this end product. It is suggested that this may be because of a change in bacterial flora or increased absorption of choline with liver injury.

The observations of these authors make significant contributions to the understanding of the metabolic fate of choline. Pursued further, they may offer important new information concerning the place of choline in the treatment of hepatic disease.

BLOOD GLUCOSE AND THE URGE TO EAT

Study of mechanisms concerned with homeostasis of blood glucose has interested physiologists since the time of Claude Bernard. Since maintenance of blood sugar ultimately depends upon food intake, the relationship between the two has been investigated. Yet, although considerable information is available concerning the influence of the diet pattern upon blood glucose levels, the possible part played by variations in blood glucose in actually conditioning energy intake has never been elucidated.

Earlier studies (A. J. Carlson, *Am. J. Physiol.* **31,** *175 (1913)*; H. D. Janowitz and M. I. Grossman, *Ibid.* **155,** *28 (1949)*) included attempts to relate blood sugar levels to hunger. However, because of excessive preoccupation with the intermediary role of the stomach or, perhaps, because of lack of appreciation of the limitations of studying absolute levels of blood sugar without appraisal of the degree of glucose utilization taking place at the time, experimental results from such studies were inconclusive.

Recently, J. Mayer and M. W. Bates (*Am. J. Physiol.* **168,** *812 (1952)*) have reported experiments concerned with the relationship between blood glucose levels and food intake in normal and hypophysectomized alloxan-treated ("Houssay-type") rats. They attempted to determine whether elevation or depression of blood sugar levels within physiologic limits could significantly influence food intake. They also compared

the effect on food intake of nutrients which were capable of exerting a direct influence on blood sugar (parenterally administered glucose and fructose) with that of preparations of similar caloric value which did not directly affect blood glucose levels. Intravenous fat emulsions and parenterally given sucrose were used as "nonglucogenic" materials.

Since alloxan-treated hypophysectomized rats clear administered glucose from the circulation very slowly, such animals were studied in order to determine whether exaggerated changes in food intake could be induced by glucose administration. The studies were carefully controlled by use of a comparable series of saline-injected animals.

The results indicated that the procedures which induced hyperglycemia without appreciably decreasing utilization of glucose were accompanied by significant reductions in food intake. A high order of statistical significance was obtained even when the data were corrected for the caloric value of the glucogenic substances. Reduction in food intake was particularly striking in the "Houssay" animals, in which the prolonged hyperglycemia was accompanied by marked anorexia. When sucrose and fat emulsions were administered, no significant influence on food intake was observed.

The authors acknowledge that there is a problem involved in applying their "glucostatic" theory of food intake regulation to conditions associated with chronically abnormal levels of blood glucose, such as diabetes mellitus. However, they point out that in the uncontrolled diabetic patient with polyphagia, glucose starvation of nervous centers concerned with food intake regulation may coexist with abnormally high levels of circulating but metabolically ineffectual glucose.

Although they imply that a quantitative relationship between carbohydrate utilization and food intake regulation may exist, the authors make no attempt to carry their thesis to this point. Whether it is technically possible to reduce this postulated relationship to quantitative terms remains an important question. Certainly, the work of H. P. Himsworth ("The Goulstonian Lectures on the Mechanism of Diabetes Mellitus," *Lancet Press, London (1939)*) has indicated that although blood glucose levels and tolerance to glucose may vary considerably, utilization of carbohydrate by peripheral tissues as reflected by the area between arterial and venous glucose tolerance curves tends to remain surprisingly constant in a given subject. In terms of the glucostatic theory, increased food intake might be regarded as one of the homeostatic responses which act to ensure that this area of the arteriovenous difference is not appreciably diminished.

The problem of the origin of the urge to eat is one of the oldest in physiology and its study is associated with many of the great names in the history of this science. In view of the many advances in our knowledge about the intermediary metabolism of foodstuffs, one wonders whether the interesting correlation between variations in blood glucose and food intake cannot now be quantitated. If this can be done, an advance in one of the most elusive problems in physiology will have been accomplished.

SPECIFICITY OF THE STREPOGENIN EFFECT ON BACTERIAL GROWTH

Of particular interest among the variety of substances which are needed to satisfy the nutritional requirements of bacteria is that one which is present in partially hydrolyzed proteins, strepogenin (*Nutrition Reviews* **4,** *273 (1946)*; **6,** *277 (1948)*; **8,** *132 (1950)*). The chemical identity of strepogenin has not been established. However, the evidence available indicates that activity relates to a peptide-like structure.

Serylglutamic acid peptides have been shown to possess growth-promoting activity for *Lactobacillus casei* (D. W. Woolley, *J. Biol. Chem.* **166,** *783 (1946)*; C.-H. de Verdier and G. Ågren, *Acta chem. Scandinav.* **2,** *783 (1948)*).

E. Kodicek and S. P. Mistry (*Biochem. J.* **51,** *108 (1952)*) of the Dunn Nutritional Laboratory at the University of Cambridge have concerned themselves with the specificity of the strepogenin effect and the distribution of this activity in biologic materials. Some interest attaches to the method of determination of strepogenin activity since the authors found it necessary to modify previously described methods of assay for the purposes of their experiments. A strain of *L. casei* was utilized as a test organism. The basal medium contained the necessary water-soluble vitamins, purines, amino acids, and salts. Energy sources were provided by inclusion of glucose and potassium acetate. Bovine crystalline insulin subjected to controlled partial acid hydrolysis was used as the strepogenin standard instead of the standard prepared from liver fractions by earlier workers. This substitution was made because of the higher activity of the insulin hydrolysate. A small bacterial inoculum of standard size was made into the sterile medium, the culture was incubated, and growth was determined by turbidimetric measurement after eighteen hours. Bacterial growth was expressed in terms of milligrams dry weight of organisms from a calibration curve relating turbidity to dry weight.

The authors observed that the addition of glutamine, L-asparagine, or L-aspartic acid to the basal medium had a small stimulatory effect on bacterial growth. These effects were less than one sixth of that produced by strepogenin. Moreover, addition of these compounds in combination produced no stimulus greater than that due to L-asparagine alone. For this reason this amino acid was included in the basal medium in all assays of strepogenin activity. With this modification there was a linear dose response curve and good agreement of results in triplicate analyses.

Having thus established satisfactory conditions for strepogenin assay the authors proceeded to determine the activity of a number of highly purified proteins and substances of biologic origin. In these assays the substances were subjected to preliminary acid hydrolysis under the conditions demonstrated to liberate maximum activity from crystalline bovine insulin. Assays were done at several concentrations so that a dose response curve could be derived and compared with that of the strepogenin standard.

Bovine insulin which had not been hydrolyzed gave only 10 per cent of the activity of that which had been acid hydrolyzed. Recrystallization and digestion with chymotrypsin did not significantly influence the strepogenin activity of bovine insulin. However, insulin from other species, *viz.*, pig and sheep, possessed activity four times that of bovine insulin. This greater potency did not seem to relate to the relative concentrations of given amino acids in the insulins of different origins. It is of interest to note that the liver fractions tested possessed only a small fraction of the activity of bovine insulin at low concentrations, where maximum activity was determined for insulin. However, at higher concentrations the strepogenin activity exceeded that of insulin.

A number of recrystallized enzymes were assayed. Pepsin and chymotrypsin had substantially greater strepogenin effect after acid hydrolysis than did the standard. Less pure preparations of these enzymes had less activity. Viruses of plant origin were tested and it was found that tobacco mosaic virus and turnip yellow mosaic virus had relatively high activity after hydrolysis. Other proteins from animal sources which demonstrated considerable strepogenin effect included myosin, lactoglobulin, and plasma albumin. A variety of other proteins were subjected to this assay and had negligible

activity. Some of these were egg albumin, wool protein, human globin, bovine hemoglobin, edestin, gelatin and casein from various sources.

These observations indicate that proteins from many sources contain strepogenin activity. It cannot be concluded that the proteins which failed to have this effect under the conditions of these experiments do not contain this activity, since it has not been shown that the procedure selected for partial hydrolysis is optimal for release of activity in all proteins, although it was demonstrated to provide maximal and reproducible release of activity from crystalline bovine insulin.

Proteins from different sources gave different strepogenin effects under the conditions of assay. Certain of these demonstrated growth response curves parallel to and are comparable to that of the standard insulin preparation. Of this type were trypsin and bovine plasma albumin. In other instances the growth response curve was decidedly different from that of insulin, usually with a significantly steeper slope. This was true of sheep insulin and liver fractions, chymotrypsin and tobacco mosaic virus. It would seem that strepogenin activity as determined in these procedures may relate to a number of different substances. The authors express the view that the bacterial growth-stimulating effect of partially hydrolyzed proteins is relatively specific, but is not possessed by a single peptide. It more likely relates to a group of peptides with a particular configuration.

Because the method of assay used in these experiments differs from that utilized by other investigators it is difficult to compare these observations with those reported earlier. However, many of the proteins described as without strepogenin activity have been found by other workers to be free of this effect. The method of assay would seem to be an improved one and it is to be hoped that it will be applied further in efforts to elucidate the nature of the bond or the chemical entity to which this apparently important function in bacterial growth attaches.

CITROVORUM FACTOR CONJUGATE

After it was discovered (H. E. Sauberlich and C. A. Baumann, *J. Biol. Chem.* **176,** *165 (1948)*) that the organism *Leuconostoc citrovorum* required an unidentified factor for growth, subsequent studies indicated that, though not identical with folacin, this factor appeared to have a more or less close metabolic relationship to that vitamin. Folacin is known to occur in both free and conjugated form, the latter yielding the free vitamin upon treatment with an enzyme preparation from hog tissues (O. D. Bird, *et al., Ibid.* **157,** *413 (1945)*). To determine whether or not the citrovorum factor exists as a conjugate, a recent study was made of the action of an enzyme preparation from hog kidney upon the citrovorum factor in dried brewers' yeast (C. H. Hill and M. L. Scott, *Ibid.* **196,** *189 (1952)*).

The yeast was autoclaved for thirty minutes and the liquid, after centrifuging, was used as the substrate. After incubation with the kidney enzyme at pH 4.5 overnight, the mixture was steamed to inactivate the enzyme and assayed for the citrovorum factor. Blank determinations showed that the kidney extract alone contained little, if any, citrovorum factor. The yeast extract before incubation contained 1200 units per gram, but after incubation it contained 3700 units per gram, indicating that the citrovorum factor in yeast exists largely in bound form. Furthermore, the behavior of the enzyme toward cysteine and hydrogen sulfide is so much like that of folacin conjugase from the same source, that the conclusion was reached that these enzymes are strikingly similar, if not identical.

Similar studies were carried out on extracts of chick liver (C. H. Hill and M. L. Scott, *J. Biol. Chem.* **196,** *195 (1952)*), using the hog kidney extract as substrate. There was a more than thirty-fold increase in free citrovorum factor after incubation with the enzyme, showing that, as in dried brewers' yeast, the citrovorum factor exists in chick livers largely as a conjugate. It was further observed that the chick liver itself contained an enzyme capable of liberating free citrovorum factor from its conjugate which is greatly stimulated in activity by ascorbic acid. This enzyme also hydrolyzed the conjugate in yeast extract. Further experiments with the chick liver enzyme demonstrated that the increased liberation of citrovorum factor from conjugate by chick liver enzyme, in the presence of ascorbic acid, is due to activation of the enzyme by the vitamin C and not to a conversion of folacin to the citrovorum factor.

NIACIN METABOLISM IN PREGNANCY

Several reports have appeared which indicate that there is an increase in the excretion of niacin metabolites in late pregnancy in women (M. C. Moore *et al., J. Am. Dietet. Assn.* **23,** *847 (1947)*; E. I. Frazier, T. Porter, and M. J. Humphrey, *J. Nutrition* **37,** *393 (1949)*; H. Oldham, B. B. Sheft, and Porter, *Fed. Proc.* **8,** *391 (1949)*).

A further investigation of the metabolism of niacin in pregnancy was recently reported by M. E. Lojkin, A. W. Wertz, and C. G. Dietz of the University of Massachusetts (*J. Nutrition* **46,** *335 (1952)*). This study is part of a cooperative project in the Northeast region dealing with the nutritional status of pregnant women. The general outline of the over-all study has been reported (*Northeast Regional Publication* **5,** *Cornell Univ. Agr. Exp. Station, Memoir 307 (1951)*).

In the recent report by Lojkin and associates urinary niacin metabolites were determined in 61 pregnant women between the ages of 16 and 34 years. Twenty-three of these were primiparae and the others were second, third, and fourth pregnancies.

The twenty-four-hour excretion of N-methylniacinamide ranged between 4.2 and 13 mg. in early pregnancy, between 6.6 and 32.9 mg. in late pregnancy, and between 1.7 and 8.7 mg. at three months post partum. These wide variations in the excretion of this niacin metabolite apparently occur generally and have been observed by many other investigators. Despite these wide variations, there was a statistically significant rise in the excretion of N-methylniacinamide with advance in pregnancy.

This increased excretion was not associated with, or could not be attributed to, increased niacin intakes or to increased tryptophan intakes. Intakes of the latter were based on calculations from food records whereas niacin intakes were calculated from food records and also measured for the twenty-four-hour periods previous to and during urine collections. In late pregnancy the average N-methylniacinamide excretion was 107 per cent of the intake, indicating either an increased synthesis or a mobilization of body stores.

The excretion of 6-pyridone was measured in 10 of the 61 subjects. The excretion of this metabolite generally paralleled the excretion of the N-methyl compound. For example, in early pregnancy, twenty-four-hour excretions of 7.1 mg. of the latter and 10.0 mg. of 6-pyridone were found, whereas in late pregnancy the excretion of the N-methyl compound had increased to 17.9 mg. and the 6-pyridone to 28.5 mg. It was thus apparent that the increased excretion of the one metabolite of niacin (the N-methyl) could not be accounted for through impairment of the oxidizing processes since the more highly oxidized derivative (6-

pyridone) was also excreted in increased amounts with advance in pregnancy.

The excretion of total niacin (acid-hydrolyzable metabolites of niacin) was also observed to increase with advance in pregnancy, although the increase was considerably less than that observed for N-methylniacinamide.

It was also noted that the excretion of niacin metabolites was significantly higher in multiparae than in primiparae.

The total excretion of 6-pyridone, N-methylniacinamide and total acid-hydrolyzable metabolites averaged more than the sum of the niacin consumed and of the niacin that could be synthesized from the tryptophan in the diet. This would suggest the conclusion that the maternal organism is drawing from niacin stores in the body during pregnancy.

The Massachusetts workers also conducted similar studies with rats where the niacin content of the diet and the total niacin intake could be determined accurately. Twenty-four pregnancies were studied, 18 in which the animals were fed ad libitum where feed intake gradually increased with advance in pregnancy, and 6 in which niacin intake was restricted to that of early pregnancy and the increased energy demand in later pregnancy was satisfied with added dextrose.

The twenty-four-hour N-methylniacinamide excretion under ad libitum feeding increased from an average of 291 micrograms before pregnancy to 552 micrograms in late pregnancy and decreased again to 336 micrograms ten to twenty days after parturition. Essentially similar results were obtained in the animals in which the niacin intake remained constant throughout the gestation period. Total acid-hydrolyzable metabolites followed essentially the same pattern. The combined excretions of total acid-hydrolyzable metabolites and of N-methylniacinamide represented about 48 per cent of the niacin intake in early pregnancy and about 70 per cent of the niacin intake in late pregnancy. These results again suggested that pregnancy and not the level of niacin intake was the predominant factor influencing the excretion of niacin metabolites.

The factors responsible for the stimulation of the excretion of niacin metabolites during pregnancy still remain unknown. Niacin is utilized for the formation of coenzymes I and II. Increased activity of these enzymes associated with embryonic development may be the source of the increased losses of the niacin metabolites in pregnancy. This is of course only speculation. The results presented by the Massachusetts workers with rats suggest that this species can serve as a useful subject in further investigations of niacin metabolism. Whether or not the results obtained could be applied to human subjects, however, remains to be determined, since it is well known that the metabolic products of niacin metabolism vary greatly between species (*Nutrition Reviews* **8,** *291 (1950)*).

LABILE METHYL SYNTHESIS

The experimental evidence for the biosynthesis of labile methyl groups has been reviewed recently and some of the details of the mechanism were set forth (*Nutrition Reviews* **10,** *27 (1952)*). Among other compounds serving as a source of the methyl group of choline, methyl alcohol, doubly labeled in the methyl group with C^{14} and with deuterium, was administered to rats subcutaneously. The evidence indicated that the carbon of the alcohol is transferred to the methyl group of choline, but that less than one third of the hydrogens administered as the methyl group of the alcohol accompanies the carbon to form the methyl group of choline. This observation suggests

that the methyl group of the alcohol is not used intact, but rather serves as a precursor of the biologically useful methyl group in choline. The precise point of biosynthesis of methyl groups, therefore, may be prior to the formation of choline, which final stage may well be one of transmethylation. It is obvious that further information regarding the enzyme systems involved is needed to complete the picture of the biosynthesis of labile methyl groups.

In a study of the factors favoring the utilization of homocystine for the growth of young rats on methionine-free diets M. A. Bennett (*Science* **110,** *589 (1949)*) observed that the apparent biosynthesis of methyl groups, as exemplified by increase in body weight, was stimulated by the presence of folacin in the diet. A recent study (W. G. Verly, J. M. Kinney, and V. du Vigneaud, *J. Biol. Chem.* **196,** *19 (1952)*) has examined the part played in labile methyl metabolism by folacin and by leucovorin, a factor known to stimulate the growth of *Leuconostoc citrovorum*.

C^{14}-labeled methanol and methanol with both C^{14} and deuterium in the methyl group were administered subcutaneously to adult rats which were maintained on experimental rations free from folacin. In some cases sulfasuxidine was added; in others, vitamin B_{12} was injected intraperitoneally, and in a third instance, the antimetabolite methyl folic acid was included in the food to retard growth. In every instance, when growth had ceased, the rats were returned to the adequate diet and the labeled methanol injections were begun. Folacin was given by mouth or by intraperitoneal injection and leucovorin was given by injection, in each case during the administration of the methanol. At the end of the experimental period (one and four-tenths to four days in different experiments) the choline was isolated from the tissues and degraded to trimethylamine, which was in turn converted to the chloroplatinate, upon which compound the determinations for C^{14} and deuterium were carried out.

From a comparison of the counts per minute per millimole in the methyl group of the trimethylamine chloroplatinate in the control animals with the value obtained from the rats receiving folacin or leucovorin, the conclusion can be drawn that the synthesis of the methyl group in choline from methanol is stimulated by both folacin and leucovorin. That these vitamins may be part of an enzyme system generally involved in labile methyl metabolism is suggested by the observation (W. Sakami and A. D. Welch, *J. Biol. Chem.* **187,** *379 (1950)*) that the synthesis of methyl groups from formate by liver slices is favored by folacin and also by the results of *in vivo* studies (J. A. Stekol, K. W. Weiss, and S. Weiss, *Fed. Proc.* **10,** *252 (1951)*) in which folacin stimulated the formation of methyl groups from formate. The manifold instances in which folacin is known to catalyze metabolic reactions emphasizes the likelihood that the biosynthesis of labile methyl groups does not involve traffic in the intact radical.

NOTES

Letter to the Editor

Dear Sir:

I am writing to comment on the review entled "Amino Acid Composition of Neoplastic Tissue" which appeared on pages 302 and 303, Volume 9, October 1951 issue of *Nutrition Reviews*.

We feel that there has been a misinterpretation of our experimental results as well as of those of Sauberlich and Baumann, referred to in this review. It appears to us incorrect to conclude that "the results of Sauberlich and Baumann may explain to some extent, at least, the differences in amino acid composition observed by Dunn and co-workers."

The facts are that Sauberlich and Baumann found no differences in amino acid composition between normal and cancerous tissues. Furthermore, they made no corrections for connective tissue, whereas Dunn *et al.* made the necessary corrections for collagen and elastin and found a significant difference between normal and tumor tissues. Therefore, our results may explain the failure of Sauberlich and Baumann to find any difference, which would not be unexpected since they did not adjust for the masking effect of amino acids in the diluting connective tissue.

M. S. DUNN
Professor of Chemistry
University of California
Los Angeles 24, California

Adrenal Hormones and Sodium Excretion

Known adrenal hormones usually affect sodium metabolism to reduce the excretion of sodium. This is particularly true of desoxycorticosterone acetate (DCA). Moreover, in Addison's disease, which is characterized by a marked deficiency of adrenocortical hormones, urinary and perhaps other excretory losses of sodium are characteristic.

Suggestive evidence that the adrenal may under certain circumstances secrete an abnormal steroid inducing sodium excretion rather than retention is presented by J. W. Jailer (*Tr. N. Y. Acad. Sci.* **13,** *262 (1951)*). Three infants with a syndrome originally described by L. Wilkins, W. Fleischmann, and J. E. Howard (*Endocrinology* **26,** *385 (1940)*) were studied. The syndrome is one of congenital adrenal hyperplasia, with symptoms and other evidence of Addison's disease. To maintain normal serum sodium concentrations it was necessary to treat the infants with DCA in addition to a dietary supplement of 5 g. of sodium chloride. Fifteen months later evidence of genital hyperplasia was observed in one infant and advanced maturation of ossification was found by x-ray. A high urinary excretion of 17-ketosteroids and normal formaldehydogenic steroids indicated excessive androgen production but normal production of 11-oxysteroids. Bilateral adrenal hyperplasia was found by perirenal air insufflation.

Further study of this patient at the age of 2½ years indicated that 5 mg. of DCA and 5 g. of supplemental sodium chloride daily were not sufficient to maintain him in sodium balance. Moreover, ACTH administration made matters worse, since his blood pressure fell, sodium excretion rose, and he looked poorly. This was in spite of evidence that his adrenals had been stimulated, as indicated by an increase in urinary 17-ketosteroids and corticoids. These data suggested to the authors that stimulation of the adrenals might be causing a diuresis of sodium rather than a retention.

It was subsequently found possible to maintain this patient on daily cortisone with the withdrawal of DCA. Moreover, this induced a fall in urinary 17-ketosteroid excretion. Three more patients without electrolyte disturbances, but with the adrenal genital syndrome, likewise demonstrated a fall in urinary 17-ketosteroid excretion when given cortisone, indicating that cortisone inhibited pituitary ACTH excretion (*Nutrition Reviews* **9,** *349 (1951)*).

The fact that DCA and sodium chloride did not maintain this individual in sodium balance and that ACTH administration led to symptoms of Addison's disease concomitant with a stimulation of the adrenal cortex, indicated to the authors that the adrenals might in fact be secreting a steroid "whose principal physiological action is one of sodium excretion rather than retention."

Yeast and Dietary Liver Injury

Two forms of pathologic change in the liver have been observed in animals given abnormal and inadequate diets: a fatty liver, and one with acute necrosis. Both may be fatal or lead to fibrosis (*Nutrition Reviews* **7,** *302 (1949)*). It is clear that the fatty livers are produced by giving food deficient in choline or its precursors. The relationship of this experimentally produced fatty liver to the disease seen in man is not yet entirely clear.

The dietary necrosis in rats has been produced by a number of different diets, which are characteristically low in protein, especially in the sulfur-containing amino acids, and deficient in vitamin E. In addition, diets in which the protein was largely yeast have been shown to be necrogenic, although not all yeasts appeared to be effective. European yeasts were highly effective in producing the lesion, whereas most American yeasts were not.

K. Schwarz, who described dietary necrosis resulting from high-yeast diets (*Z. f. physiol. Chem.* **283,** *187 (1948)*), has examined the differences in the yeasts in an effort to understand better the genesis of dietary liver necrosis. Two studies are reported. In the first (*Proc. Soc. Exp. Biol. Med.* **77,** *818 (1951)*), it was demonstrated that necrosis could be produced in 100 per cent of animals fed a vitamin E-free diet to which torula yeast had been added. This yeast was tried since it had been grown on sulfite liquor instead of corn steep liquor or other cereal products, and thus might be toxic or deficient because of the difference in the medium used for growth. The other American yeasts which were grown on cereal liquors did not induce dietary necrosis. Vitamin E added to the diet completely prevented the necrosis while L-cystine, although moderately effective in this regard, was not completely successful even when large amounts were added to the diet.

The disease was characterized by widespread areas of necrosis in the liver which the author considered were precipitated just before death and were the reason for sudden death. Ulcers of the forestomach were seen in approximately half the rats developing dietary necrosis, and renal damage was found in almost 90 per cent. Areas of hemorrhage in the gastrointestinal tract and in the mediastinal lymph nodes were also observed.

Further study by the same investigator (*Proc. Soc. Exp. Biol. Med.* **78,** *852 (1951)*) demonstrated that the yeasts which did not produce the lesions were also protective when added to the diet containing torula. For example, when 10 per cent of a cultured dried yeast was added to the 30 per cent torula ration, the incidence of liver necrosis fell from 100 per cent to approximately 30 per cent. The same or better could be done with a debittered dried brewers' yeast added to the diet.

The protective effect of these two yeasts could not be attributed to cystine and vitamin E because of the small content of these substances in the yeasts added and the lack of difference in the content of these compounds between the torula and the protective yeast.

The author believed from these studies that there was another factor in the protective yeasts than cystine and vitamin E which was water-soluble and stable against acid hydrolysis. This he called factor 3, 1 and 2 being cystine and vitamin E. Thus, the absence of three factors was considered necessary for the production of dietary hepatic necrosis.